

### **REMARKS**

Claims 46-60 are currently pending in the application. Claim 47 are amended. Claims 46, 47, 50, and 51 are allowed. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

#### **Formal Matters**

The Examiner indicated to Applicants' representative by telephone on February 2, 2005 that the claims contain several terms, specifically "2MeSADP" and "ADPbetaS" which are unclear as they are inconsistent with the manner in which these compounds would be referred to in the art. The Examiner suggested that Applicants look at the scientific literature to determine the most appropriate was to designate these two ADP analogs. Applicants submit that they have searched the MEDLINE database using the terms 2MeSADP and ADPbetaS and identified 41 and 158 hits, respectively. Applicants submit herewith a sample of abstracts (Exhibits A-E) which indicate the apparent art-recognized designation of these compounds; that is, the terms 2MeSADP and ADPbetaS are found throughout the literature and are listed exactly as they appear in the instant claims. Accordingly, Applicants are unclear as to where the Examiner finds inconsistency with the use of the terms in the art, and to the extent that the Examiner maintains the position that amendment is necessary, Applicants request further clarification.

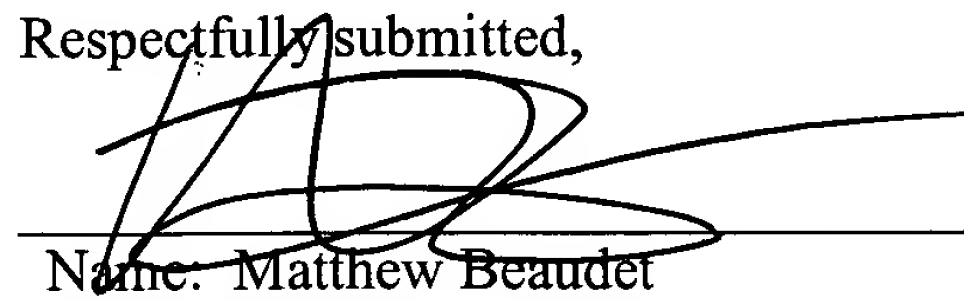
#### **Rejection of Claims 48, 49, 52-60 Under 35 U.S.C. §112, Second Paragraph**

The Examiner had rejected claims 48, 49, and 52-60 under §112, second paragraph as being indefinite for failing to explicitly recite a the concentration of the recited compound and candidate modulator. The Examiner indicated to Applicants' representative by telephone on February 1, 2005, however, that the rejection of claims 48, 49, and 52-60 under §112, second paragraph is being withdrawn. Applicants thank the Examiner for his careful reconsideration of the instant claims, and submit that the rejection is now moot.

Applicants submit that in view of the foregoing remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the claims of the present invention.

Date: February 7, 2005

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Matthew Beaudet', is written over a horizontal line.

Name: Matthew Beaudet

Registration No.: 50,649

Customer No.: 29933

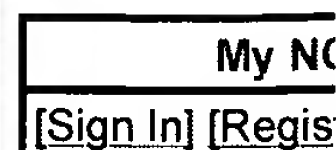
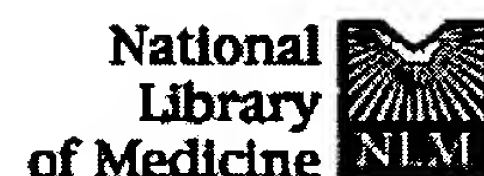
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☐ 1: J Thromb Haemost. 2004 Nov;2(11):1980-8.

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## Adenine triphosphate nucleotides are antagonists at the P2Y receptor.

**Kauffmanstein G, Hechler B, Cazenave JP, Gachet C.**

INSERM U.311, Etablissement Francais du Sang-Alsace, Strasbourg, France

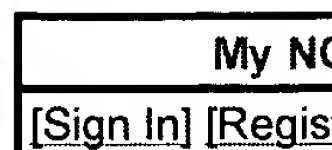
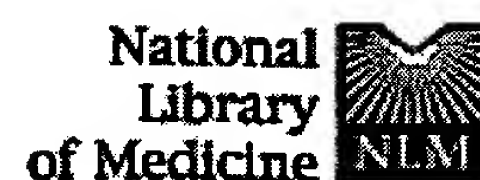
The aim of the present study was to characterize the pharmacological profile the P2Y(12) receptor for several adenine triphosphate nucleotides in view of their possible roles as partial agonists or true antagonists. Two distinct cellular systems were used: P2Y(1) receptor deficient mouse platelets (platelets) previously shown to express a native and functional P2Y(12) receptor and 1321 N1 astrocytoma cells stably expressing the human P2Y(12) receptor (1321 N1 P2Y(12)). ADP and its structural analogues inhibited cAMP accumulation in dose-dependent manner in both platelets and 1321 N1 P2Y(12) cells with a similar rank order of potency, 2 methylthio-ADP (2MeSADP) >> ADP - Adenosine 5'-(beta-thio) diphosphate (AlphaDPbetaS). Commercial ATP, 2-chloro; ATP (2ClATP) and 2 methylthio-ATP (2MeSATP) also inhibited cAMP accumulation in both cell systems. In contrast, after creatine phosphate (CP)/creatine phosphokinase (CPK) regeneration, adenine triphosphate nucleotides lost their agonistic effect on platelets and behaved as antagonists ADP (0.5 microm)-induced adenylyl cyclase inhibition with IC(50) of 13.5 +/- 4.8, 838 +/- 610, 1280 +/- 1246 microm for 2MeSATP, ATP and 2ClATP, respectively. In 1321 N1 P2Y(12) cells, CP/CPK regenerated ATP and 2ClATP lost their agonistic effect only when CP/CPK was maintained during the cAMP assay. The stable ATP analogue ATPgammaS antagonized ADPbetaS-induced inhibition of cAMP accumulation in both platelets and 1321 N1 P2Y(12) cells. Thus, ATP and its triphosphate analogues are not agonists but rather antagonists at the P2Y(12) receptor expressed in platelets or transfected cells, provided cAMP is taken to remove diphosphate contaminants and to prevent the generation of diphosphate nucleotide derivatives by cell ectonucleotidases.

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☐ 1: J Pharmacol Exp Ther. 2004 Dec;311(3):1038-43. Epub 2004 Sep 02.

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### Induction of novel agonist selectivity for the ADP-activated P2Y receptor versus the ADP-activated P2Y12 and P2Y13 receptors conformational constraint of an ADP analog.

Chhatrivala M, Ravi RG, Patel RI, Boyer JL, Jacobson KA, Harden TK

University of North Carolina, School of Medicine, Department of Pharmacology, CB #7365, Chapel Hill, NC 27599-7365, USA.

ADP is the cognate agonist of the P2Y1, P2Y12, and P2Y13 receptors. With goal of identifying a high potency agonist that selectively activates the P2Y1 receptor, we examined the pharmacological selectivity of the conformational constrained non-nucleotide analog (N)-methanocarba-2MeSADP [(1'S,2'R,3'S,4'R,5'S)-4-[(6-amino-2-methylthio-9H-purin-9-yl)-1-diphosphoryloxymethyl]bicyclo[3.1.0]hexane-2,3-diol] among the three ADP activated receptors. Each P2Y receptor was expressed transiently in COS-7 cells, and inositol lipid hydrolysis was quantified as a measure of receptor activity. In the case of the Gi-linked P2Y12 and P2Y13 receptors, a chimeric protein, Galphaq/i, was coexpressed to confer a capacity of these Gi-linked receptors to activate phospholipase C. 2MeSADP (2-methylthio-ADP) was a potent agonist at all three receptors exhibiting EC50 values in the sub to low nanomolar range. In contrast, whereas (N)-methanocarba-2MeSADP was an extremely potent (EC50=1.2 +/- 0.2 nM) agonist at the P2Y1 receptor, this nucleotide analog exhibited no agonist activity at the P2Y12 receptor and very low activity at the P2Y13 receptor. (N)-Methanocarba-2MeSADP also failed to block the action of 2MeSADP at the P2Y12 and P2Y13 receptors, indicating that the (N)-methanocarba analog is not an antagonist at these receptors. The P2Y1 receptor selectivity of (N)-methanocarba-2MeSADP was confirmed in human platelets where it induced the shape change promoted by P2Y1 receptor activation without inducing the sustained platelet aggregation that requires simultaneous activation of the P2Y12 receptor. These results provide the first demonstration of a high-affinity agonist that discriminates among the three ADP-activated P2Y receptors, and therefore, introduce a potentially important new pharmacological tool for delineation of the relative biological action of

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these three signaling proteins.

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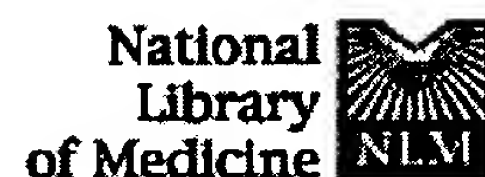
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☐ 1: J Neurochem. 2004 Apr;89(2):442-53.

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## P2Y<sub>12</sub> receptor stimulation inhibits beta-adrenergic receptor-induced differentiation by reversing the cyclic AMP-dependent inhibition of protein kinase B.

Van Kolen K, Slegers H.

Laboratory of Cellular Biochemistry, Department of Biomedical Sciences, University of Antwerp, Wilrijk-Antwerpen, Belgium.

Cyclic AMP-dependent induction of differentiation by activation of the beta-adrenergic receptor is correlated with inhibition of protein kinase B activity concomitant with growth arrest and increase in glial fibrillary acidic protein (GFAP) synthesis in rat C6 glioma cells. Costimulation of the beta-adrenergic receptor with purinergic receptors activated by 2-methylthio-adenosine-5'-diphosphate (2MeSADP) increased protein kinase B (PKB) phosphorylation above the level measured in non-stimulated cells and abolished cAMP-dependent differentiation. Transfection of cells with constitutively active PK confirmed that reactivation of PKB is involved in the 2MeSADP-dependent inhibition of GFAP synthesis. The P2Y<sub>12</sub> and P2Y<sub>13</sub> receptor antagonist AR-C69931MX [N(6)-(2-methylthioethyl)-2-(3,3,3-trifluoropropylthio)-beta,gamma-dichloro-methylene ATP] decreased PKB phosphorylation to the level in non-stimulated cells, whereas the P2Y<sub>13</sub> antagonists pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) and P(1),P(3) (adenosine-5') tetraphosphate (Ap(4)A) did not alter the 2MeSADP-induced phosphorylation of PKB, showing that enhanced PKB activity and subsequent phosphorylation of glycogen synthase kinase-3 is due to stimulation of the P<sub>2</sub>Y<sub>12</sub> receptor. In addition, experiments in the presence of pertussis toxin and phosphatidylinositol 3-kinase (PI 3-K) activity assays demonstrated that the P2Y<sub>12</sub> receptor-mediated increase in PKB phosphorylation is G(i) protein- and PI 3-K-dependent. The presented data demonstrated that a cAMP-dependent inhibition of PKB induces differentiation of C6 glioma cells and the inhibition of adenylate cyclase and reactivation of the PI 3-K/PKB pathway by the P2Y<sub>12</sub> receptor reverses differentiation into enhanced proliferation.

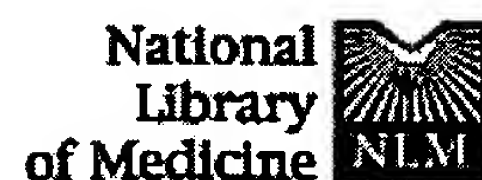
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☐ 1: Glia. 2005 Jan 18; [Epub ahead of print]

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## Metabotropic P2 receptor activation regulates oligodendrocyte progenitor migration and development.

Agresti C, Meomartini ME, Amadio S, Ambrosini E, Serafini B, Franchi L, Volonte C, Aloisi F, Visentin S.

Department of Cell Biology and Neurosciences, Istituto Superiore di Sanita, Rome, Italy.

To gain insights into the role of purinergic receptors in oligodendrocyte development, we characterized the expression and functional activity of P2 receptors in cultured rat oligodendrocyte progenitors and investigated the effects of ATP and its breakdown products on the migration and proliferation of this immature glial cell population. Using Western blot analysis, we show that oligodendrocyte progenitors express several P2X (P2X(1,2,3,4,7)) and P2Y (P2Y(1,2,4)) receptors. Intracellular Ca(2+) recording by Fura-2 video imaging allowed to determine the rank potency order of the P2 agonists tested:

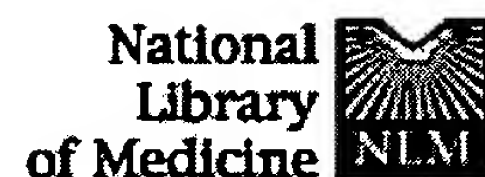
ADPbetaS ≈ ADP = Benzoyl ATP > ATP > ATPgammaS > UTP, alpha,beta meATP ineffective. Based on the above findings, on pharmacological inhibition by the antagonists oxATP and MRS2179, and on the absence of alpha,beta meATP-induced inward current in whole-cell recording, P2X(7) and P2Y(1) were identified as the main ionotropic and metabotropic P2 receptors active in OPs. As a functional correlate of these findings, we show that ATP and, among metabotropic agonists, ADP and the P2Y(1)-specific agonist ADPbetaS, but not UTP, induce oligodendrocyte progenitor migration.

Moreover, ATP and ADP inhibited the proliferation of oligodendrocyte progenitors induced by platelet-derived growth factor, both in purified culture and in cerebellar tissue slices. The effects of ATP and ADP on cell migration and proliferation were prevented by the P2Y(1) antagonist MRS2179. By confocal laser scanning microscopy, P2Y(1) receptors were localized in NG2 labeled oligodendrocyte progenitors in the developing rat brain. These data indicate that ATP and ADP may regulate oligodendrocyte progenitor function by a mechanism that involves mainly activation of P2Y(1) receptors. (c) 2005 Wiley-Liss, Inc.

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☐ 1: Diabetes. 2004 Dec;53 Suppl 3:S63-6.

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## P2Y purinergic potentiation of glucose-induced insulin secretion and pancreatic beta-cell metabolism.

Farret A, Vignaud M, Dietz S, Vignon J, Petit P, Gross R.

Center for Pharmacology and Health Biotechnology, CNRS UMR 5160, Faculte de Medecine, Institut de Biologie, 4 Boulevard Henri IV, CS89508, 34960 Montpellier Cedex 2, France.

Purine nucleotides and their analogs increase insulin secretion through activation of pancreatic beta-cell P2Y receptors. The present study aimed at determining the role of glucose metabolism in the response to P2Y agonists and whether ATP-activated K<sup>+</sup> channels (KATP channels) are involved in this response. The experiments were performed in the rat isolated pancreas, perfused with a Krebs-bicarbonate buffer supplemented with 2 g/l bovine serum albumin under dynamic glucose conditions from 5 mmol/l baseline to 11 mmol/l. ADPbetaS (0.5 micromol/l) was selected as a stable and selective P2Y agonist. This compound, ineffective on the 5 mmol/l glucose background, induced a significant threefold increase in insulin release triggered by the glucose challenge. The effect of ADPbetaS was markedly reduced ( $P < 0.001$ ) in the presence of an inhibitor of glucose metabolism. In addition to glucose, the ADPbetaS analog also amplified the beta-cell insulin response to 15 mmol/l methyl pyruvate ( $P < 0.05$ ), but it was ineffective on the insulin response to 2.5 mmol/l methyl succinate. A nonmetabolic stimulus was applied using tolbutamide (1 micromol/l). Insulin secretion induced by the KATP channel blocker was strongly reinforced by ADPbetaS ( $P < 0.001$ ), which prompted us to check a possible interplay of KATP channels in the effect of ADPbetaS. In the presence of diazoxide 250 micromol/l and 21 mmol/l KCl, ADPbetaS still amplified the second phase of glucose-induced insulin secretion ( $P < 0.001$ ). We conclude that P2Y receptor activation is able to promote insulin secretion through a mechanism, involving beta-cell metabolism and a rise in intracellular calcium, and this effect does not result from a direct inhibitory effect on KATP channels.

PMID: 15561924 [PubMed - in process]

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